II. ~~AMENDMENTS TO SPECIFICATION~~

Please insert the following replacement paragraphs at the identified pages and lines.

Please amend the paragraph at page 3, lines 27-34, as indicated below:

Since proteinases and their receptors and inhibitors seem to play a pivotal role in the basic mechanisms leading to cancer invasion, these molecules may be expressed at a very early time point in the carcinogenic process. As many of these molecules exert their biological action extracellularly, they may be present at elevated levels in body fluids, even in patients with early stage invasive malignant disease. Moreover, since these molecules are involved in the more basic features of malignant progression, e.g. invasion and metastasis, it should be investigated ~~whether~~ which forms of cancer that display an increase in these molecules.

Please amend the paragraph at page 6, lines 13-28, as indicated below:

In Example 4, which includes data regarding plasma TIMP-1 levels from healthy blood donors and from patients with known colorectal cancer, it is shown that patients suffering from colorectal cancer have significantly elevated total TIMP-1 levels in their preoperative plasma samples. A percentile plot of the total TIMP-1 levels in plasma from all colorectal cancer patients and from healthy blood donors shows that a total TIMP-1 concentration of 119.1 μg/L is the 90^{th} centile of the healthy donors. Using this cut-off, 68% of the colorectal cancer patients were identified as having elevated plasma TIMP-1 levels. When analyzing the colon cancer patients , it was shown that total TIMP-1 measurements in plasma identified 75% of the colon cancer patients (~~diagnostic~~ sensitivity) with a 90 specificity (10% of the healthy blood donors were classified as being high). Similarly, it was shown for the rectal cancer patients ~~alone~~, that total TIMP-1 measurements in plasma identified 60% of the rectal cancer patients with a 90% ~~diagnostic~~ specificity. If a higher or lower sensitivity or specificity is desired, the cut-off value can be changed. This is illustrated in Figure 13 showing ROC curves of total TIMP-1 in plasma from colorectal cancer patients. In addition, ROC curves are included for the individual groups of colon and rectal cancer patients. Any other information which can be derived from these ROC curves falls within the scope of the present invention.

Please amend the paragraph at page 7, lines 4-10, as indicated below:

The clinical value of a marker for cancer detection or screening is related to its ability to detect early stages of disease, potentially impacting survival. It was shown that total TIMP-1 was as efficient in detecting or screening ~~a screening marker in~~ early stage colorectal cancer (Dukes' stages A and B) as it was in the total population of colorectal

cancer patients (Figure 14), Thus, ~~screening~~ detection or screening with total TIMP-1 will result in more patients being diagnosed with early stage cancer. In a similar manner, any information that can be derived from Figure 14 falls within the scope of the present invention.

Please amend the paragraph at page 7, lines 17-23, as indicated below:

The specificity of a given cancer detection or screening test is based on the efficiency of the test to identify only those patients suffering from cancer while patients suffering from non-malignant diseases should not be identified as false positive subjects. In the case of colorectal cancer, it is important that the test in question can distinguish between malignant and non-malignant diseases of the colon and rectal. This is particularly important for diseases like Crohn's disease and ulcerative colitis, since patients with these diseases are at higher risk of developing cancer.

Please amend the paragraph at page 7, lines 25-34, as indicated below:

In Example 5 it is shown that total TIMP-1 levels are significantly higher in patients with colorectal cancer than in patients with inflammatory bowel diseases (IBD), showing that total TIMP-1 can be used to detect or screen colorectal cancer in a population of patients with IBD. That TIMP-1 is not increased in non-malignant diseases is supported by a recent paper, (Keyser *et al*, 1999), demonstrating that patients with rheumatoid arthritis do not have increased plasma TIMP-1 levels. Also, by comparing total TIMP-1 levels among patients with IBD (excluding patients with clinically assessed acute active disease, n=4) and healthy blood donors, no significant differences in total plasma TIMP-1 levels were found (p=0.56), showing that these non-malignant diseases do not give false positive test results.

Please amend the paragraph at page 8, lines 17-18, as indicated below:

In Example 8, the use of the TIMP-1:MMP-9 complex assay as an aid for the detection or screening ~~diagnosis~~ of colorectal cancer is described.

Please amend the paragraph at page 8, lines 20-24, as indicated below:

TIMP-1 is known to exist either as the free molecule or in complex with MMP's, preferentially MMP-9. Measuring total TIMP-1, complexed TIMP-1 and free TIMP-1 will make it possible to validate each of these species for their potential detection or screening ~~diagnostic~~ value. In addition, it will be possible to calculate ratios or any derived

algorithm between the different species which might provide additional detection or screening ~~diagnostic~~ value.

Please amend the paragraph at page 8, lines 26-27, as indicated below:

In Example 9, the detection or screening ~~diagnostic~~ value of the combination of total and free TIMP-1 is described.

Please amend the paragraph at page 9, lines 5-12, as indicated below:

In Example 10, total plasma TIMP-1 values in preoperative blood samples from a cohort of 322 patients with primary breast cancer (stage I and II) as compared with total TIMP-1 levels in 108 healthy blood donors are described. It was shown that the breast patients had a median, total TIMP-1 level of 88.3 μg/L, while the healthy donors had a median plasma concentration of total TIMP-1 of 88.9 μg/L. The difference between these values is not clinically significant, supporting the specificity of TIMP-1 levels for the detection or screening ~~diagnosis~~ of colorectal cancer. However, it should be studied whether elevated plasma TIMP-1 levels are found in patients with early stage non-colorectal cancer.

Please amend the paragraph at page 9, lines 24-29, as indicated below:

TIMP-2 is another tissue inhibitor of metalloproteinases with a high degree of homology to TIMP-1. Using a specific immunoassay for TIMP-2, concentrations of this inhibitor were determined in plasma samples from colorectal cancer patients and in healthy blood donors. No significant differences in plasma TIMP-2 levels were found between the two populations, supporting the unique value of TIMP-1 as an aid for the early detection or screening ~~diagnosis~~ of colorectal cancer.

Please amend the paragraph at page 25, line 12, as indicated below:

Detection or screening ~~diagnostic~~ value of total TIMP-1 in patients with colorectal cancer.

Please amend the paragraph at page 27, lines 1-3, as indicated below:

The median TIMP-1 level in plasma from healthy donors was 88.6 μg/L with a range of 1.0-156.2 μg/L. There was a highly significant statistical difference in the total plasma TIMP-1 values between the colorectal cancer patients and the healthy blood donors.

Please amend the paragraph at page 27, lines 5-10, as indicated below:

The median total TIMP-1 value for the 64 colorectal cancer patients was 138.2 μg/L (range: 80.7-790.6 μg/L). Stratifying the patients into colon and rectal cancer, the median, total TIMP-1 values were 152.2 μg/L (range: 80.7-626.2 μg/L) for colon and 133.6 (range: 84.3-790.6) for rectal cancer. There was a highly significant statistical difference in the total plasma TIMP-1 values between the colon and rectal cancer patients each compared with the healthy blood donors.

Please amend the paragraph at page 27, lines 12-20 as indicated below:

Detection or screening ~~diagnostic~~ value of total TIMP-1

Using the measured total TIMP-1 levels in plasma from healthy donors and the 591 colorectal cancer patients, Receiver Operating Characteristics (ROC) curves were generated to evaluate the ~~diagnostic~~ detection or screening value of total TIMP-1. As seen in Figure 13, the ROC curve was initially steep, indicating a high sensitivity and specificity of total TIMP-1 as a marker for colorectal cancer. It appears that the AUC is greater for colon cancer than for rectal cancer. Figure 14 shows a similar ROC curve now including only patients with early stage colorectal cancer, i.e. Dukes' stage A or B disease. Also shown is the ROC curve for early stage (Dukes' stage A and B) right-sided colon cancer.

Please amend the paragraph at page 27, lines 21-25 as indicated below:

Using the total TIMP-1 levels in plasma from healthy donors and in the second cohort of 64 colorectal cancer patients, ROC curves were again constructed to confirm the detection or screening ~~diagnostic~~ value of TIMP-1. As seen in Figure 15, the curve was again initially steep, indicating a high sensitivity and specificity of total TIMP-1 as a marker for colorectal cancer.

Please amend the paragraph at page 28, lines 2-11 as indicated below:

These data suggest that total TIMP-1 measurements in plasma can be used as a detection or screening procedure to aid in identifying patients with a high risk of having colorectal cancer. In particular, total TIMP-1 was as effective in identifying patients with early cancer (Duke's stage A+B) as identifying patients with more advanced disease. Also, total TIMP-1 was even more effective in identifying patients with early stage, right-sided colon cancer, a procedure ~~diagnosis~~ that is difficult with conventional ~~diagnostic~~ detection or screening procedures. Right-sided colon cancer cannot be visualized by flexible sigmoidoscopy, a standard colon cancer screening methodology. It has a more insidious onset than do left-sided lesions, and clinical symptoms develop only in late

stage disease. Early detection ~~diagnosis~~ or screening of right sided colon cancer has the potential to reduce the mortality of this disease.

Please amend the paragraph at page 28, lines 12-15 as indicated below:

Moreover, the smaller, prospective trial corroborated the results of the larger retrospective study, further confirming the detection or screening ~~diagnostic~~ value of total TIMP-1 in patients suffering from colorectal cancer.

Please amend the paragraph at page 29, lines 19-20 as indicated below:

Detection or screening ~~diagnostic~~ value of total TIMP-1 in combination with CEA in patients with colorectal cancer.

Please amend the paragraph at page 30, line 6 as indicated below:

Detection or screening ~~diagnostic~~ value of total TIMP-1 and CEA

Please amend the paragraph at page 30, lines 13-19 as indicated below:

When the total TIMP-1 values from Example 4 are included together with CEA, the sensitivity of the marker combination was found by logistic regression analysis to be 52%. The additional ~~diagnostic~~ sensitivity obtained by the addition of CEA measurements in serum is highly significant ($p<0.0001$). When stratifying the patient cohort into colon and rectal cancer, the sensitivity was 61% and 39%, respectively at the 98% specificity level. Including only patients with right-sided colon cancer, the sensitivity was 74%. A graphical illustration of these results appears from Figure 17.

Please amend the paragraph at page 30, lines 22-28 as indicated below

These data show that by adding an additional marker, an improvement in the ~~diagnostic~~ sensitivity of total TIMP-1 can be obtained, while maintaining a high specificity of 98%. Thus, the combination of CEA and TIMP-1 could be useful as a detection or screening ~~screening~~ procedure to identify patients with a high risk of having colorectal cancer. In particular, this combination was efficient in identifying patients with early stage cancer (Duke's stage A+B). Also, this combination was highly effective in identifying patients with early stage, right-sided colon cancer.

Please amend the paragraph at page 30, line 31 as indicated below:

Lack of detection or screening ~~diagnostic~~ screening value of plasma free TIMP-1 levels in patients with colorectal cancer.

Please amend the paragraph at page 31, line 20 as indicated below:

Lack of detection or screening ~~diagnostic~~ value of free TIMP-1

Please amend the paragraph at page 31, lines 21-22 as indicated below:

Figure 18 shows the ROC curves generated from the plasma measurements of free TIMP-1. The AUC is 0.61 when determining the ~~diagnostic~~ detection or screening performance of free TIMP-1.

Please amend the paragraph at page 31, lines 25-26 as indicated below:

These data show that free TIMP-1 alone is not likely to be useful as a screening or detection marker to identify patients with a high risk of having colorectal cancer.

Please amend the paragraph at page 31, lines 29-30 as indicated below:

Detection or screening ~~Diagnostic~~ value of TIMP-1:MMP-9 complex measurements in patients with colorectal cancer.

Please amend the paragraph at page 31, lines 31-page 32, line 2 as indicated below:

TIMP-1:MMP-9 complex levels in plasma from colorectal cancer patients and in plasma from age and gender matched healthy individuals can be measured using the TIMP-1:MMP-9 assay described in Example 2. TIMP-1:MMP-9 complex values from healthy donors and cancer patients can be compared and the detection or screening ~~diagnostic~~ value determined.

Please amend the paragraph at page 32, lines 4-7 as indicated below:

Using the measured values of free, total, and TIMP-1:MMP-9 complex levels, the detection or screening ~~diagnostic~~ value of ratios or fractions can be calculated. In addition other molecules e.g. serum CEA can be added to these calculations to generate mathematical algorithms to increase the overall detection or screening ~~diagnostic~~ value.

Please amend the paragraph at page 32, lines 10-11 as indicated below:

Detection or screening ~~Diagnostic~~ value of the combination of plasma total TIMP-1 and plasma free TIMP-1 in patients with colorectal cancer

Please amend the paragraphs at page 33, lines 17-28 as indicated below:

Using the total and the free TIMP-1 levels in plasma from healthy donors and from the 64 colorectal cancer patients, ROC curves were constructed for each of these TIMP-1 forms. The total TIMP-1 values obtained confirm the detection or screening ~~diagnostic~~ value of total TIMP-1 measurements in patients with colorectal cancer . As seen in Figure 15, the curve was again initially steep, indicating a high sensitivity and specificity of total TIMP-1 as a marker for colorectal cancer. The ROC curve for free TIMP-1 was discussed in Example 7.

The corresponding data for total TIMP-1 in this patient population gave an AUC of 0.88. However, an increase in the detection or screening ~~diagnostic~~ value was obtained when combining free and total TIMP-1 (AUC=0.94). This increase is highly statistically significant, $p<0.0001$ and shows the value of the important embodiment of using both free and total TIMP-1 in the same analysis.

Please amend the paragraph at page 33, lines 31-33 as indicated below:

These data suggest that the combination of total TIMP-1 and free TIMP-1 measurements in plasma can be used as a screening or detection procedure to aid in identifying patients with a high risk of having colorectal cancer.

Please amend the paragraph at page 34, lines 1-3 as indicated below:

In a similar manner as described in the present example, other ratios and/or combinations or mathematical permutations between free and total TIMP-1 values can be calculated and used for detection or screening ~~diagnostic~~ purposes.

Please amend the paragraphs at page 34, lines 5-9 as indicated below

Calculations of relationships among all the various forms of TIMP-1, including total and free TIMP-1, total concentration of complexes (total TIMP-1 – free TIMP-1), and/or the concentration of TIMP-1:MMP-9, might be extremely useful in the management, and especially distinguishing ~~the differential diagnosis~~ of patients with non-malignant diseases ~~and~~ from patients with cancer.

Please amend the paragraphs at page 34, lines 12-13 as indicated below:

Lack of detection or screening ~~diagnostic~~ value of total TIMP-1 measurements in patients with primary (stage I and II) breast cancer.

Please amend the paragraphs at page 34, lines 26-29 as indicated below:

These data demonstrate that patients with primary breast cancer have total TIMP-1 values that are not significantly different from those of healthy blood donors. Thus, these data support the specificity of TIMP-1 measurements in the detection or screening ~~diagnosis~~ of patients with colorectal cancer.

Please amend the paragraphs at page 34, lines 32-33 as indicated below:

Detection ~~Diagnostic~~ value of plasma total TIMP-1 in patients with metastatic (stage IV) breast cancer.

Please amend the paragraphs at page 36, line 1 as indicated below:

Detection or screening ~~Diagnostic~~ value of TIMP-2 measurements in patients with colorectal cancer

Please amend the paragraph at page 36, lines 14-16 as indicated below:

These data support the specificity of TIMP-1 measurements in the ~~diagnosis~~ detection or screening of patients with colorectal cancer, supporting the unique value of TIMP-1 as an aid in the early detection ~~diagnosis~~ of colorectal cancer.

Please amend the paragraph at page 37 as indicated below:

ABSTRACT

The present invention describes a method for determining whether an individual is likely to ~~suffering~~ suffer from cancer by determining a parameter representing the TIMP-1 concentration in body fluid samples from the individual.